JOURNAL OF VIROLOGY, Sept. 1979, p. 677-684
0022-538X/79/09-0677/08$02.00/0

Vol. 31, No. 3

Measles Virus-Specified Polypeptide Synthesis in Two Persistently Infected HeLa Cell Lines

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Received for publication 25 April 1979

Measles virus-directed protein synthesis was examined in two HeLa cell lines (K11 and K11A) that are persistently infected with wild-type measles virus. Four viral proteins (H, hemagglutination protein; P, nucleocapsid-associated protein; NP, the major nucleocapsid protein; and M, the matrix protein) were readily detected in both cell lines by immune precipitation of [^{35}S]methionine-labeled cell extracts followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, three (H, NP, and M) of the four viral proteins in both K11 and K11A cells differed from the corresponding viral proteins synthesized in HeLa cells acutely infected with the parental wild-type virus. In addition, the M protein from K11A cells migrated significantly more slowly on sodium dodecyl sulfate-polyacrylamide gel electrophoresis than the M protein from K11 cells, and there appeared to be slight differences in the H and NP proteins between these two persistently infected cell lines. The altered viral proteins detected in K11 and K11A cells appeared to be the result of viral mutations rather than changes in the host cell, since virus recovered from these cells directed the synthesis of similar aberrant viral proteins in HeLa cells. Virus recovered from K11 cells and virus recovered from K11A cells were both temperature sensitive and grew more slowly than wild-type virus. HeLa cells infected with virus recovered from K11 cells readily became persistently infected, resembling the original persistently infected K11 cells. Thus, viral mutations are associated with persistent measles virus infections in cell cultures.

Measles virus has been implicated in the slowly progressive neurological disease subacute sclerosing panencephalitis (SSPE) (1, 3, 10, 20, 23, 32). For this reason, as well as the more tentative link of measles virus to multiple sclerosis (2, 15, 16, 20), interest has emerged concerning the possible mechanism(s) of measles virus persistence both in vivo and in cell culture. Although the ultimate understanding of the mechanism of measles virus persistence in human hosts will require an understanding of a variety of factors, including host genetic and immunological mechanisms, persistently infected cell cultures provide a useful model for studying this problem.

Several laboratories have established cell lines persistently infected with measles virus (4, 5, 12, 17-20, 22, 26). The first of these lines were developed by R. Rustigian in the early 1960s (26-28) and subsequently have been well characterized. After acute infection of HeLa cells with the Edmonston strain of measles virus, a small number of cells survived, giving rise to a population of persistently infected cells. The clonal cell line K11 was isolated from this original population and is a prototype for persistently infected carrier cultures that yield low levels of infectious measles virus. A second clonal line, K11A-HG-1 (hereafter referred to as K11A) was obtained after extensive serial passage of K11 in the presence of antimeasles antibody. Infectious virus cannot be detected in cell lysates or by infectious center assays of K11A cultures. However, infectious virus has been recovered after extensive cocultivation with Vero cells (29). K11A is thus a prototype for nonyielding persistently infected cell lines.

By comparing the persistently infected K11 and K11A cells with HeLa cells acutely infected with the wild-type (wt) virus used to initiate these persistent infections, it is possible to study measles virus persistence. Previous studies have indicated that viral antigens (26-28) and virus-specific RNA (38) are present in cells of both the

K11 and K11A prototype lines. We have previously noted that the M proteins found in these persistently infected cells migrate more slowly in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) than the wt M protein (36, 37). This finding has been demonstrated recently in another laboratory (25). In the present report, we extended these observations on the measles virus polypeptides synthesized in K11 and K11A cells by using immune precipitation of cell extracts followed by SDS-PAGE. Differences in several of the virus-specific polypeptides were found. The viral matrix (M) protein in these persistently infected cell lines not only had a slower mobility on SDS-PAGE than the parental wt virus M protein, but the mobilities of the M proteins from K11 and K11A cells also differed from each other. In addition, SDS-PAGE revealed alterations in the hemagglutinin (H) proteins and the nucleocapsid (NP) proteins found in K11 and K11A cells compared with the H and NP proteins found in cells infected with wt virus. All of these changes resulted from viral rather than host cell mutations since the virus recovered from these cells directed the synthesis of similar aberrant H, NP, and M proteins during acute infection of normal cells.

MATERIALS AND METHODS

Cell lines. Parental HeLa cells, the persistently infected HeLa clones K11 (27) and K11A (28), and the cured virus-free lines K11-VF and K11A-VF were maintained as previously described (27). K11 cells were originally isolated approximately 20 years ago (27). During this interval, the cells used in this study were frozen, stored, and reestablished in culture a total of four times. The cells were stored in the frozen state for a total of approximately 14 years. During the remaining 5.5 to 6 years, the cells were maintained in culture and were passaged 150 to 170 times. The current studies were performed on K11 cells removed from storage approximately 9 to 15 months previously. These cells had been frozen at passage 109. The K11A cells were isolated from K11 cells (28) and were maintained in culture for 26 passages. This took approximately 1 year. They were then frozen in liquid nitrogen and were thawed after 12 years. They were then grown and maintained for an additional 1 to 6 months in culture and studied between passage 30 and passage 50. CV-1 cells were maintained as previously described (35). The virus-free cured subclone K11-VF (passages 10 to 15) was isolated from K11 cells as an individual colony of cells that had spontaneously lost its persistent phenotype as shown by viral immunofluorescence (VIF) (38). The K11A-VF subline was obtained after extensive subcultivation at a low density of persistently infected K11A cells at passage 34. The subcultivation consisted of seeding 10^6 K11A cells in a Blake bottle with a surface area of 140 cm^2. The cells were then serially subcultured at the same density. After 47 serial passages measles virus immunofluorescence was no longer detected. The K11A-VF subline was studied at passage 53.

Virus. Stocks of parental Edmonston measles virus (wt) were prepared by infecting roller bottle cultures containing 5×10^7 Vero cells with virus at an input multiplicity of infection (MOI) of 0.01 PFU/cell. After the development of marked cytopathic effects (3 to 6 days), virus was harvested by scraping the cells into 10 ml of culture fluid; this was followed by homogenization with a Teflon homogenizer as previously described (27). Cell debris was removed by centrifugation at $1,500 \times g$ for 10 min. The supernatant was stored at −85°C. K11-virus was recovered from homogenates of K11 cells (passage 153). K11A-virus was recovered from K11A cells (passage 30) after cocultivation with Vero cells (29). Stocks of K11-virus and K11A-virus were prepared as described for wt virus after three serial passages of K11-virus and six serial passages of K11A-virus in Vero cells.

Biological parameters. The analysis of intracellular and surface fluorescent viral antibody staining was performed as previously described (27). Hemadsorption was measured from the amount of hemoglobin estimated from E_{413} by the method of Katzman and Wilson (11). Infectious center assays using viable cells and plaque assays using homogenized cell lysates were performed as previously described (29).

Infection of cells for polypeptide analysis. Confluent cell monolayers in Falcon multicluster wells (catalog no. 3008; Falcon Plastics; 10^5 cells per well) were infected with virus at an MOI of 50 PFU/cell. After 2 h of adsorption at 37°C in a 5% CO_2 atmosphere, the infected monolayers were fed with 1 ml of IMEMZO (Richter improved minimal essential medium; Associated Biomedical Systems) supplemented with 10% fetal calf serum and returned to 37°C.

[^{35}S]methionine labeling of intracellular viral polypeptides. Infected or uninfected cell monolayers were washed twice in 1 ml of methionine-free Eagle minimal essential medium and preincubated for 1 h in 1 ml of the same medium. [^{35}S]methionine incorporation was carried out as described previously (35) in 0.5 ml of methionine-free medium containing 100 μCi of [^{35}S]methionine. [^{35}S]methionine labeling was performed at 16 to 18 h postinfection for wt virus and at 22 to 24 h postinfection for K11-virus and K11A-virus. Incorporation was terminated by rinsing the monolayers twice with ice-cold 0.01 M Tris–0.0015 M MgCl–0.14 M NaCl, pH 7.2.

Preparation of antimeasles antiserum. A total of 5×10^6 CV-1 cells acutely infected with wt virus were disrupted by freeze-thawing and mixed with an equal volume of complete Freund's adjuvant. Rabbits were injected intramuscularly at 2-week intervals and bled 2 weeks after the third injection. The rabbit serum was extensively absorbed with uninfected CV-1 and HeLa cells before use.

Immune precipitation. After the termination of [^{35}S]methionine incorporation, the cells from each well (10^5 cells) were suspended on 0.5 ml of lysing buffer (0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 0.01 M Tris-hydrochloride, pH 7.4) and passed through a 26-gauge needle three times. Debris was removed by centrifugation at $2,000 \times g$ for 2 h. The clarified cell extracts (~0.5 ml) were then incu-

bated for 3 h at 4°C with 50 μl of the rabbit antiserum prepared against wt measles virus. A 50-μl amount of Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals) in lysing buffer was added, and incubation continued for 45 min with frequent mixing. Immune complexes were pelleted by centrifugation at 2,000 × *g* for 2 min. The pellets were washed three times by resuspending in fresh lysing buffer and repelleting. The final pellet was suspended in 0.5 ml of gel sample buffer (14). Equal 10-μl samples were run on each gel lane.

SDS-PAGE. SDS-PAGE of proteins was carried out by the method of Laemmli (14) in a 10% polyacrylamide slab gel at a constant current of 45 mA for 2.5 h. Gels were fixed, dried, and exposed to film as previously described (35).

Efficiency of plating. Efficiency of plating was calculated as the ratio of plaques produced at 40.3°C to plaques produced at 33°C. CV-1 cell monolayers in 60-mm tissue culture dishes were infected with 0.1 ml of serial 10-fold dilutions of virus. After 2 h of adsorption at 33°C in a 5% CO_2 atmosphere, the monolayers were overlayed with medium (IMEMZO) containing 1% agar. Identical sets of plates were incubated at 33 and 40.3°C in a 5% CO_2 atmosphere. At 1 day before the plaques were counted, the infected monolayers were overlayed with agar medium containing neutral red at a final concentration of 0.006%. Plaques were counted on day 6. Plates were reincubated and monitored until day 14, with no change in the number of plaques.

Yield. Confluent CV-1 cell monolayers in 2-dram flat-bottomed screw-capped vials (Wheaton) were infected with the appropriate measles strains at an MOI of 2. After 2 h of adsorption at 33°C, the infected monolayers were washed twice in IMEMZO, fed with 1 ml of IMEMZO, and then incubated in a water bath at either 33.0 ± 0.1 or 40.3 ± 0.1°C. Samples were harvested by freezing the vials at −70°C. Titers were determined at 33°C on CV-1 cell monolayers by using the standard agar-neutral red overlay method described above.

RESULTS

Biological parameters of the prototype persistently infected and virus-free cell lines. Several biological parameters of the K11, K11A, K11-VF, and K11A-VF cell lines were monitored at the same passage level that was subsequently used for the examination of intracellular viral polypeptides in these cell lines (Table 1). In homogenized lysates of K11 cultures, the average yield of infectious virus per cell was less than 1% of that from HeLa cells acutely infected with wt virus. In addition, only 0.3% of the K11 cells, as measured by infectious centers, appeared to be producing infectious virus at any time. No infectious virus could be detected in K11A cells, either from cell lysates or by infectious center assays. The levels of hemadsorption and VIF in K11 cells were similar to, or slightly lower than, those in acutely infected cells. On the other hand, K11A cells exhibited little or no hemadsorption and significantly less surface VIF than did K11 cells, although they retained similar levels of intracellular VIF. The virus-free lines K11-VF and K11A-VF had no detectable VIF or hemadsorption activity.

TABLE 1. *Biological properties of persistently infected HeLa cells*[a]

Cells	% of cells showing immunofluorescence		Hemadsorption (E_{413}/ml per 10^5 cells)	% infectious centers	PFU/cell
	Surface	Intracellular			
HeLa-wt	96	97	0.250	ND[b]	15.6
K11	80	92	0.501	0.29	0.046
K11A	48	90	0.063	<0.05[c]	<0.005[c]
K11-VF	0	0	0.004	<0.05	<0.005
K11A-VF	0	0	0.000	<0.05	<0.005
HeLa	0	0	ND	ND	ND

[a] K11, K11A, K11-VF, and K11A-VF cells were monitored for surface and intracellular VIF, hemadsorption ability, percent infectious centers, and number of PFU per cell as described in the text. Cultures of parental HeLa cells (HeLa) and parental HeLa cells acutely infected with parental wt virus at an MOI of 180 at 30 h postinfection (HeLa-wt) were included as controls.

[b] ND, Not done.

[c] Below detectable limits.

Analysis of measles virus polypeptides in HeLa cells. Measles virus polypeptides can be detected by SDS-PAGE of [^{35}S]methionine-labeled HeLa cells infected at a high MOI (35). However, this method has not lent itself to the analysis of the persistently infected K11 and K11A cell lines since the amount of viral polypeptides found in these cell lines is lower than that found in acutely infected HeLa cells. Thus, although faint viral polypeptide bands are occasionally detected by direct SDS-PAGE of K11 and K11A cell extracts, the viral polypeptides usually cannot be detected above the cellular background (data not shown). In order to readily visualize the measles virus proteins synthesized in K11 and K11A cells, we employed immune precipitation of cell extracts, followed by SDS-PAGE.

Four major intracellular viral polypeptides (H, the surface glycoprotein responsible for hemagglutination [80,000 daltons]; P, a nucleocapsid-associated phosphoprotein [70,000 daltons]; NP, the major nucleocapsid protein [60,000 daltons]; and M, the matrix protein [37,000 daltons]) (31, 33–35) are all readily seen after immune precipitation of extracts of HeLa cells acutely infected with wt virus (Fig. 1). The F_1 and F_2 polypeptides that comprise the fusion protein (8) are not usually detected in cell extracts by the methods employed here. The same four viral polypeptides (H, P, NP, and M) are also detected in K11 and K11A cell extracts by this

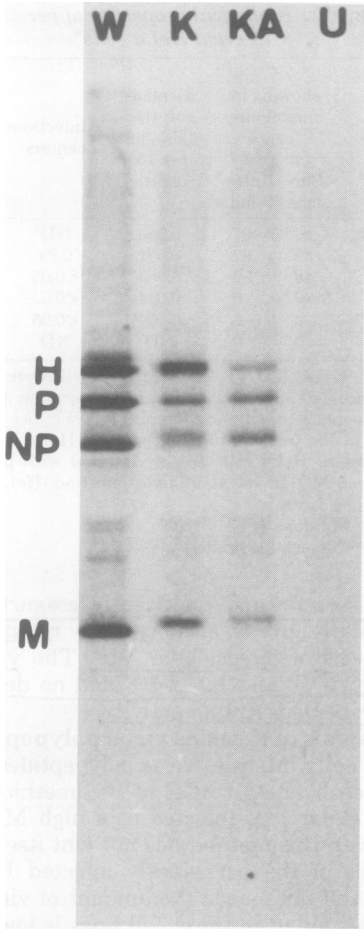

FIG. 1. *Viral polypeptides in cells persistently infected with measles virus. [^{35}S]methionine-labeled cells were immune precipitated with antimeasles antisera as described in the text. Equal samples (representing ~4 × 10^3 cells) of immune precipitates were subjected to SDS-PAGE, and the gel was exposed to film for autoradiography as described in the text. Viral proteins: H, hemagglutination glycoprotein; P, phosphoprotein associated with nucleocapsid; NP, major nucleocapsid protein (phosphorylated); M, matrix protein. Lane W, HeLa cells acutely infected with wt virus as described in the text; lane K, K11 cells; lane KA, K11A cells; lane U, uninfected HeLa cells.*

technique, although in reduced amounts. Of the viral proteins detected in K11 and K11A cells, only the P proteins appear to migrate identically on SDS-PAGE to their wt counterparts (Fig. 1).

As we have previously noted, the M proteins from both K11 and K11A cells migrate more slowly on SDS-PAGE than does the M protein from wt virus-infected cells (36, 37) (Fig. 1). In addition, the K11A cell M protein appears to migrate slightly more slowly than the K11 cell M protein.

The wt H protein appears as an intense band with a diffuse, less intense trailing band (Fig. 1), possibly representing a more highly glycosylated form of this glycoprotein (similar to that of the vesicular stomatitis virus glycoprotein [13]). This trailing band is missing in K11 cells, although the major band is as intense as that of the wt H protein. In addition, the K11 cell H band appears slightly broader than the major wt H band. The H band from K11A cells is fainter than either the K11 or the wt H protein band and appears to be relatively reduced in amount when compared with the amounts of P and NP (see below). In addition, similar to the H protein of K11 cells, the K11A cell H protein does not have a trailing band. This is also seen when the K11A lane is intentionally overloaded to produce an H band equal in intensity to the wt H band (data not shown).

The K11 cell NP protein appears as a broader band than the wt NP protein, whereas the K11A NP protein always appears as a sharp band (Fig. 1). Both the K11 and the K11A NP proteins appear to migrate slightly more slowly than the wt NP protein. Although the findings of aberrant H and M proteins from K11 and K11A cells are highly reproducible, the finding of non-wt-appearing K11 and K11A NP proteins is variable. Nevertheless, in the majority of experiments the K11 and K11A cell NP proteins appeared as in Fig. 1. Thus, the M, H, and NP proteins of K11 and K11A cells all appear to differ from the proteins of the parental wt virus. In addition, the M, H, and NP proteins in K11A cells differ from the corresponding proteins in K11 cells, the cell line from which the K11A cells were derived.

Amount of viral proteins. The lanes in Fig. 1 all represent material immune precipitated from equal numbers of cells. Less [^{35}S]methionine-labeled viral proteins appear to have been immune precipitated from K11 and K11A cells than from wt virus-infected cells. Since reduced quantities of measles viral proteins are also detected in K11 and K11A cells without immune precipitation (i.e., by direct SDS-PAGE of cell extracts [Wechsler, unpublished data]), the overall reduction in the amount of viral proteins immune precipitated from K11 and K11A cells was most likely due to an actual reduction in the amount of [^{35}S]methionine-labeled viral proteins present in these cells. The relative amounts of the individual viral proteins detected in K11 and K11A cells compared with wt virus-infected cells also appeared to differ. However, detailed studies will be needed before the absolute quantities of the K11, K11A, and wt viral proteins present in infected cells are known with certainty.

Viral polypeptides synthesized in parental HeLa cells, cured HeLa cells, or CV-1 cells by wt virus or by virus recovered from

K11 or K11A cells. To determine whether the aberrant viral polypeptides produced in K11 and K11A cells are a result of viral mutation rather than host cell mutation, we studied the synthesis of viral polypeptides directed by virus rescued from K11 cells (K11-virus) and from K11A cells (K11A-virus). The viral polypeptides synthesized by K11-virus and K11A-virus during primary infection of HeLa cells or CV-1 cells, a second cell line permissive for wt virus, were similar to those found in K11 and K11A cells (data not shown). Specifically the K11-virus and K11A-virus M, H, and NP proteins all appeared to differ from the wt virus proteins and from each other as noted for the proteins in K11 and K11A cells. In addition, both K11-VF and K11A-VF cells acutely infected with wt virus synthesized viral proteins that were completely normal (i.e., wt) in appearance (data not shown), suggesting that cellular mutation was not the cause of the altered mobilities. Thus, the aberrant electrophoretic mobilities of the viral polypeptides seen in K11 and K11A cells were due to mutations in the viruses present in these cell lines.

Biological characteristics of K11-virus and K11A-virus. The biological properties of the K11- and K11A-viruses were studied to determine whether they have, in addition to the biochemical alterations, biological properties that differ from those of the parental Edmonston strain. Compared with the parental wt virus, both K11-virus and K11A-virus are temperature sensitive, as determined by efficiency of plating and virus yield (Table 2 and Fig. 2). In addition, even at permissive temperature, both viruses grow more slowly than wt virus, reaching their peak yields at approximately 3 to 4 days postinfection, compared with 2 days postinfection for wt virus.

It is of particular interest that normal HeLa cells infected with K11-virus undergo little or no cytopathic effects and within several weeks after infection become indistinguishable from the original persistently infected K11 cells. Thus, K11-virus has a greatly enhanced capacity to produce persistently infected cell cultures.

TABLE 2. *Temperature sensitivity of K11-virus and K11A-virus efficiency of plating at permissive versus nonpermissive temperatures*[a]

Virus	Grown in:	Yield (PFU/ml) at:		EOP
		33°C	40.6°C	
wt	Vero	8.0×10^6	3.8×10^6	0.48
	CV-1	6.9×10^6	5.1×10^6	0.74
K11	Vero	6×10^7	<10	$<1.67 \times 10^{-7}$
	CV-1	1×10^6	<100	$<10^{-4}$
K11A	Vero	2×10^7	1.6×10^3	8.0×10^{-5}
	CV-1	1.3×10^5	<100	$<1.3 \times 10^{-3}$

[a] Viral stocks of wt virus, K11-virus, and K11A-virus were grown in both Vero and CV-1 cells at 33°C. Plaque assays were all performed in CV-1 cells. Efficiency of plating (EOP) for each viral stock was determined as the titer at 40.6°C divided by the titer at 33°C.

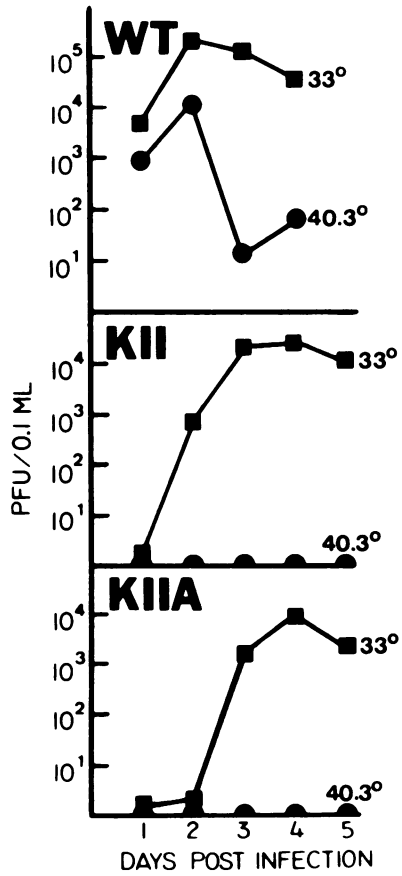

FIG. 2. *Temperature sensitivity of K11-virus and K11A-virus; viral yield at permissive and nonpermissive temperatures. Parental wt virus, K11-virus, and K11A-virus, each at an MOI of 2 PFU/cell, were grown in CV-1 cells at either 33 or 40.3°C as described in the text. Samples were taken at the times indicated, and virus production was assayed at permissive temperature (33°C) as described in the text.*

DISCUSSION

This report describes the viral polypeptides synthesized in two HeLa cell lines persistently infected with the Edmonston strain of wt measles virus. The K11 line produces low levels of infectious virus, whereas the nonyielding K11A line, derived from K11, does not produce any directly detectable, infectious virus. We have been able to readily detect the four major intracellular measles virus polypeptides (H, P, NP, and M) in both persistently infected cell lines by

using immune precipitation of extracts of [^{35}S]-methionine-labeled cells followed by SDS-PAGE.

Since cultures of K11 cells contain a high percentage of cells with intracellular and surface antigens, it is not surprising that viral polypeptides are present in these cells in readily detectable amounts. The reduced percentage of K11A cells showing surface antigen and the striking reduction in the ability of K11A cells to hemadsorb erythrocytes are consistent with the biochemical finding of reduced amounts of protein. In particular, the H and M viral polypeptides known to associate with cell membranes (31) appear to be present in K11A cells in greatly reduced amounts. Both cell lines contain viral proteins that differ significantly on SDS-PAGE from the proteins of the parental Edmonston virus and differ slightly from each other. These differences are seen in the H, NP, and M proteins. The H proteins recovered from both persistently infected cell lines do not contain the diffuse band found just above the main H band of the wt virus-induced H protein. The H protein in K11 cells is an intense band, appearing slightly broader than the wt H protein main band, whereas the H protein in K11A cells is a sharp, very faint band. The NP proteins from K11 and K11A cells also appear to differ, migrating more slowly than the wt NP. Major alterations are also seen in the M proteins; the M protein from K11 cells migrates more slowly than the M protein from wt virus-infected cells, whereas the M protein from K11A cells is the slowest migrating species.

Viral mutations rather than cellular mutations are responsible for the altered migrations of viral polypeptides seen in K11 and K11A cells since HeLa cells and CV-1 cells infected with K11 and K11A viruses synthesize aberrant proteins similar to those present in K11 and K11A cells. In a similar fashion K11 and K11A cells that had been cured of their persistent infection and subsequently infected with wt virus contained normal wt viral proteins.

In addition to these biochemical differences, certain altered biological properties indicate significant differences in the measles viruses rescued from the persistently infected cells. In particular, the K11 virus is less lytic, tends to produce persistent infection with increased efficiency, and is temperature sensitive. Preliminary experiments with K11A-virus indicate that it may not produce persistently infected cell cultures with any greater efficiency than wt virus. However, the need to extensively cocultivate K11A-virus from the nonyielding K11A cells in order to obtain isolates has made it difficult to be certain that the K11A-virus is not significantly different from its in vivo state. Studies with the K11A-virus have therefore been limited.

The multiple changes found in the proteins of measles viruses isolated from persistently infected cells strongly suggest the presence of multiple viral mutations. Accumulation of multiple viral mutations has been seen with other viruses during the course of persistent infections. For example, analysis of genomic RNA from vesicular stomatitis virus during persistence has revealed marked changes in the RNA sequence, as determined by oligonucleotide fingerprints (9). In addition, Preble and Youngner have summarized the findings of several systems in which temperature-sensitive mutants are isolated from cells persistently infected with vesicular stomatitis virus (24). Unfortunately, early passage levels of K11 and K11A cells do not exist. Thus, it is not possible to determine the order in which the observed viral mutations occurred, nor is it possible to determine which, if any, of these mutations correspond in time to the establishment of persistence. Thus, at present both the relationship of specific viral mutations to persistence and the precise nature of these mutations are not known.

Since point mutations usually are not reflected as alterations in the electrophoretic mobility of proteins, the detection of altered mobilities in three of the four viral proteins seen in K11 and K11A cells suggests that the virus in these persistently infected cells has undergone a more extensive genetic change. In cells persistently infected with vesicular stomatitis virus, most of the viral mutations appear to have accumulated over an extended period of time (9). The same may be true of cells persistently infected with measles virus. It is likely that a wide variety of mutations can be tolerated in persistently infected cells, because in contrast to lytic measles viruses that require all functions for efficient growth, viruses found in persistently infected cells do not require all viral functions. In fact, the major requirements for maintaining a persistent infection are that the virus replicate while not reverting to a lytic form, and that the cell line does not become spontaneously cured of the persistent infection. Viruses that revert to a lytic form will not survive because the persistently infected cells are resistant to superinfection and will therefore not support the growth of the lytic virus. Similarly, if a percentage of cells in persistently infected lines become spontaneously cured, these cured cells will be reinfected either by virus present in the medium or by adjacent persistently infected cells. Thus, as long as the virus has retained enough genomic information to replicate, the persistently in-

fected state can be maintained. In addition, accumulation of multiple viral mutations might be favored during persistent infection since, compared with single mutations, multiple mutations would greatly reduce the possibility of the virus reverting to a lytic form.

The finding that persistence of measles virus in cell cultures is associated with multiple mutations resulting in an altered migration of several viral proteins suggests that these mutations could occur in vivo and possibly play a role in persistence. We have previously reported that five SSPE isolates contained M proteins that migrated more slowly than the M protein of Edmonston measles virus (36, 37). A similar finding has also been reported by Schleuderberg et al. (30). In addition, Mountcastle and Choppin (21) were unable to detect any differences in migration among the M proteins of four wt measles virus strains. Furthermore, Hall et al. (6) reported that the mRNA's for the M proteins of SSPE viruses are larger than the mRNA's for the M proteins of wt viruses. Taken together, these findings suggested a possible role for an altered M protein in SSPE. However, recent studies in our laboratory (Wechsler, unpublished data) have indicated that occasional wt measles virus isolates contain M proteins with electrophoretic mobilities differing from that of the Edmonston strain M protein. Thus, the aberrant M protein is not an absolute biochemical marker for SSPE. It is of interest that sera from patients with SSPE have markedly reduced ability to precipitate the M protein of both wt and SSPE viruses (7; S. L. Wechsler, H. L. Weiner, and B. N. Fields, J. Immunol., in press). In addition, Hall et al. (6) have noted a lack of immunological cross-reactivity between the M proteins of one SSPE strain and one wt measles virus strain. Whether the mutations leading to altered migration of the M protein of measles virus are related to these results and, furthermore, whether they play a role in the genesis of SSPE and persistent infection are currently unknown. Further studies on the nature of the specific mutations responsible for the alterations of each of the viral proteins occurring during persistence in vivo, and in vitro, coupled with analysis of host immunity, may help clarify the factors responsible for persistence of measles virus.

ACKNOWLEDGMENTS

We thank Robert F. Ramig and Howard L. Weiner for their helpful critical reading of this manuscript. The expert technical assistance of Elaine Freimont and the secretarial support of Susan L. Pevear are gratefully acknowledged.

This work was supported by the National Multiple Sclerosis Society grant RC991-B-4 and by the Veterans Administration Research Program. K.C.S. is a Public Health Service predoctoral trainee under grant 5T32-GM.0719603 from the National Institutes of Health.

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